DETECTION OF CBS GENE POLYMORPHISM AND ITS RELATION TO HOMOCYSTEINE LEVEL IN VITILIGO

Thesis

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By

Sherin Mohamed Shafik ELgohary

M. B. B. ch

Supervised by

Prof. Dr. Samar Mohamed Ragaie EL-Tahlawi

Professor of Dermatology

Faculty of Medicine

Cairo University

Dr. Dalia Mohamed Abdel Halim

Assistant professor of Dermatology

Faculty of Medicine

Cairo University

Prof. Dr. Olfat Jamil Shaker

Professor of Biochemistry and Molecular Biology

Faculty of Medicine

Cairo University

Faculty of Medicine

Cairo University

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List of Abbreviations

ACE	Angiotensin converting enzyme
AD	Alzeihemer'disease
adoMet	S-adenosyl-L-methionine
AI	Autoimmune
AIS	Autoimmune susceptibility locus
APECED	Autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia
APS	Autoimmune polyendocrinopathy syndrome
BD	Behcet's disease bHcy
BFGF	Basic fibroblastic growth factor
cAMP	Cyclic adenosine monophoshate
CAT	Catalase
CBS	Cystathionine -β- synthase
CD	Cluster of differentiation
cGMP	Cyclic guanosine monophosphate
CL	Cardiolipin
CLA	Cutaneous lymphocyte-association antigen
COMT	Catechol-O-methyltransferase
CRB	C-reactive protein
CTLA	Cytotoxic T lymphocyte antigen
CVD	Cardiovascular disease
DC	Dendritic cells
DVT	Deep venous thrombosis
EDTA	Ethylene diamine tetra-acetic acid
EDTA	Ethylenediamine tetraacetate
ELISA	Enzyme-linked immunosorbent assay
ESR	Estrogen receptor
ET	Endothelins

fHcy	Free homocysteine
FOXD3	Forkhead box D3
GCH	GTP-cyclohydrolase
GFR	Glomular filtration rate
GM-CSF	Granulocyte monocyte- Colony stimulating factor
Gr-B	Granzyme
GV	Generalized vitiligo
H_2O_2	Hydrogen peroxide
Hcy	Homocysteine
HLA	Human leucocyte antigen
HPLC	High performance liquid chromatography
IBD	Inflammatory bowel disease
ICAM	Intracellular adhesion molecules
IDDM	Insulin dependent diabetes mellitus
IFN	Interferon
Ig	Immunoglobulins
IL	Interleukin
KD	Kilodalton
LC	Langerhans cells
LV	Livedoid vasculpathy
MCHR	Melanin-concentrating hormone receptor
MHC	Major histocompatibility complex
MOP	Methoxypsoralen
MS	Methionine synthase
MSH	Melanocyte stimulating hormone
MTHF	Methyl tetrahydrofolate
MTHFR	Methylenetetrahydrofolate reductase enzyme
MV	Mixed vitiligo
MYG	Melanocyte proliferating gene
N5, N10- MTHF	N5, N10-methylenetetrahydrofolate

NA	Nucleic acid
NB-UVB	Narrow band-Ultraviolet B
NF	Nuclear factor
NF-Y	Nuclear factor Y
NKC	Natural killer cells
NO	Nitric oxide
NSV	Nonsegmental vitiligo
NTD	Neural tube defect
OS	Oxidative stress
P value	Probability value
P	Petit (short arm)
P5P	Pyridoxal -5- phosphate
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PD	Parkinson disease
PGE2	Prostaglandin E2
PLP	Pyridoxal-phosphate
Pmel	Melanosomal matrix protein
PMEL	Melanocyte protein or premelanosome protein
PTPN22	Protein tyrosine phosphatase non- receptor 22
PUVA	Psoralen and ultraviolet A
Q	Long arm
ROS	Reactive oxygen species
RP	Raynaud's phenomenon
SAH	S- adenosyl homocysteine
SAM	S- adenosyl methionines
SBI	Silent brain infarction
Scc	Squamous cell carcinoma
SCF	Stem cell factor
SLE	Systemic lupus erythematosus

SNP	Single nucleotide polymorphism
Sp1/Sp3	Specificity protein 1/3
SV	Segmental vitiligo
Th1	T-helper cell 1
THcy	Total homocysteine
TNF	Tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
Tregs	Regulatory T cells
TRP	Tyrosinase-related protein
TYRP	Tyrosinase related protein
UV	Ultraviolet
VASI	Vitiligo activity score index
VDR	Vitamin D receptor
VIDA	Vitiligo disease activity
VNTR	Variable number tandem repeats

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Abstract

Background: Vitiligo is an acquired, progressive, multifactorial, disorder of the skin and mucous membranes. An elevated Hcy level has been described in vitiligo. Multiple cofactors are needed for the metabolic pathways of Hcy such as vitamin B_{12}, vitamin B_6, folate, 5,10 methylenetetrahydrofolatereductase enzyme (MTHFR) and Cystathionine -β-synthase (CBS) enzyme.

Aim of work: The aim of the present study is to detect CBS gene polymorphism and its relation to homocysteine level in vitiligo.

Patients and methods: This observastional case control study was conducted on 100 patients with vitiligo and 80 age and sex matched controls. From each A 5ml blood sample was taken for detection of CBS (844ins68) gene polymorphism by PCR and detection of Hcy level by Axis Homocysteine Enzyme Immunoassay kit.

Results: A significant higher frequency of the CBS (844ins68) gene polymorphism (homozygous or heterozygous) was found in the patient group in relation to the control group (25% vs 12.5% with P = 0.035*). A statistically significant difference was found between cases with normal genotype and those with mutant genotype as regards family history (p = 0.000*). Also a statistically significant difference was found between cases and controls as regards Hcy level (p = 0.000*).

However no statistically significant difference was found between cases with normal genotype and those with mutant genotype as regards the age (P = 0.506.), extent (P = 0.655), age of onset (P = 0.097), duration of illness (P = 0.179), VIDA (P = 0.412),VASI (p = 0.905) and Hcy level (p = 0.403). A significant correlation was found between Hcy level and age of patients, disease duration, extent of disease and VASI score p (0.033), (0.021) , (0.000), (.000) respectively. Non significant correlation was found between Hcy level and CBS (844ins68) genotypes and VIDA (p = 0.232), (p = 0.103) respectively.

Conclusion: We concluded that CBS gene polymorphism may paly an additional role in the susceptibility of individuals for vitiligo.

Key words: Vitiligo, polymorphism, Cystathionine -β- synthase, Homocysteine.

Introduction

Vitiligo is a disorder of pigmentation characterized by the presence of depigmented skin macules due to chronic and progressive loss of melanocytes from the cutaneous epidermis. Large population surveys have shown a worldwide incidence of 0.5%-2% with the disease beginning before the age of 20 in 50% of cases **(Rezaei et al., 2007).**

Vitiligo susceptibility is not believed to be sex-linked, but 6-38% of patients have family members with the disease indicating a hereditary factor. However, the inheritance pattern of the disorder is consistent with a polygenic trait not transmission by simple Mendelian mechanism **(Rezaei et al., 2007).**

The etiology of vitiligo is unknown and several hypotheses (including autoimmune, neural, radical, self-destruction and inherent defect theories) have been proposed to explain the pathogenesis of this disorder **(Namian et al., 2009).**

There are several arguments that vitiligo is a genetically dependent disease due to presence of familial cases and cases reported in twins, also there is a high risk for both children and siblings of a subject with vitiligo **(Lacour and Ortonne, 1995).**

Genetic studies have demonstrated that certain genes are crucial for the development of vitiligo; these genes either cause the disease or increase its susceptibility. One speculation would be that one or more genes are responsible for premature death of melanocytes. Genes affecting melanocyte growth either directly or via paracrine factors (genes coding for keratinocyte melanotrophic factor and the combined effect of genes

controlling autoimmune phenomenon could be involved **(Lacour and Ortonne, 1995).**

Homocysteine (Hcy) is a non-essential sulfur containing amino acid produced during the conversion of methionine to cysteine in all cells. Multiple cofactors are needed for the metabolic pathways of Hcy such as vitamin B_{12}, vitamin B_6, folate and 5,10 methylenetetrahydrofolate reductase enzyme (MTHFR), Cystathionine -β-synthase (CBS) enzyme. Thus deficiency of these cofactors may cause hyperhomocysteinaemia **(Ricart et al., 2006).**

It is possible that an increase of local Hcy interferes with normal melanogenesis and plays a role in the pathogenesis of vitiligo **(Shaker and EL Tahlawi, 2008)**. Several theories may explain the possible effects of elevated Hcy on melanocytes in vitiligo: The production of toxic reactive oxygen species by Hcy oxidation **(Guilland et al., 2003)**. Homocysteine inhibits tyrosinase enzyme probably by interaction with copper at the active site of the enzyme **(Reish etal., 1995)**. A possible genetic association between the genetic determinant of plasma Hcy level and the susceptibility to vitiligo may be involved **(Souto et al., 2005)**

Cystathionine-β-synthase, also known as CBS, is an enzyme that is encoded by the CBS gene. It catalyzes the first step of the trans-sulfuration pathway, from homocysteine to cystathionine **(Meier et al., 2001).**

The association of CBS gene polymorphism and elevated Hcy level has been described (**Ale´ssio et al., 2008).** Cystathionine-β-synthase (CBS) deficiency is the most common genetic cause of severe hyper-homocysteinaemia. The CBS gene is located on chromosme 21 **(Avramopoulos et al., 1993).** It has two types: Homozygous form

(congenital Homocystinuria) which is an autosomal recessive trait is associated with high Hcy up to 400 μmol/L and methionine concentrations **(Mudd et al., 2000)** and Heterozygous form: The significance of this trait is less clear, People who are heterozygous for CBS deficiency have normal fasting Hcy levels in 30% to 50% of cases **(Lentz et al., 2000)**.

Aim of the Work

The aim of the present study is to detect CBS gene polymorphism in vitiligo patients and its possible association with homocysteine level.